

## **REMARKS**

### **Status of the Claims**

Claims 1-3, 5-8, 10, 12, 14-29, 31-35, and 37-39 are pending in the present application.

Claims 8 and 14-27 are withdrawn as directed to a non-elected invention. Claims 1, 28, and 29 are amended to cancel subject matter or to correct antecedent basis. Accordingly, no new matter is added by way of this amendment. Reconsideration is respectfully requested.

### **Objections to the Claims**

Claim 29 is objected to for the informalities set forth on page 2 of the outstanding Office Action. This objection is respectfully traversed.

The Examiner suggests that claim 29 be amended to specify “increasing the number of CD-8-positive cells in a population of cytotoxic lymphocytes”, *see Office Action*, page 2.

Applicants have amended claim 29 according to the Examiner’s recommendation. Thus, it is respectfully requested that this objection be withdrawn.

### **Issue under 35 U.S.C. §112, second paragraph**

Claims 28-29 and 38-39 are rejected under 35 U.S.C. § 112, second paragraph, for improper antecedent basis due to the recitation of “the expanded cytotoxic lymphocytes”, *see Office Action*, page 2. This rejection is respectfully traversed.

Applicants have amended claims 28 and 29 to specify “the cultured cytotoxic lymphocytes.” Accordingly, Applicants believe the rejection is overcome and respectfully request withdrawal.

### **Issues under 35 U.S.C. § 112, first paragraph**

Claims 1-3, 5-7, 10, 12, 28-29, 31-35, and 37-39 are rejected under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement, *see Office Action*, pages 3-5. Applicants respectfully traverse.

#### *Basis for the Rejection*

The Examiner acknowledges that the claimed methods are enabled for Peripheral Blood Mononuclear Cells, (“PBMC’s”). However, the Examiner asserts that the instant specification

fails to adequately support the use of NK cells, umbilical cord blood mononuclear cells, hematopoietic stem cells and blood components containing these cells with the claimed methods.

In particular, the Examiner indicates that the use of umbilical cord blood mononuclear cells for differentiation into a cytolytic lymphocyte population with fibronectin and IL-2 is unpredictable. To support this contention, the Examiner cites Lucivero *et al.*, *Int. J. Clin. Lab. Res.*, 1996, 26:255-261, ("Lucivero"), which allegedly teaches that umbilical cord blood lymphocytes are different in phenotype and function from lymphocytes of normal adults and display a functionally immature phenotype. The Examiner further asserts that Lucivero teaches that anti-CD3 stimulants fail to induce proliferation of cord blood lymphocytes. Accordingly, the Examiner asserts that differentiation of cord blood lymphocytes into a population of cells comprising enhanced cytolytic activity would be highly unpredictable.

*The amended claims*

Independent claims 1, 28, and 29, are amended to specify that the precursor cells are peripheral blood mononuclear cells, umbilical cord blood mononuclear cells, and blood components containing these cells. The amended claims do not describe NK cells or hematopoietic stem cells. Accordingly, this aspect of the rejection is moot.

*An ordinary artisan would have recognized from the present specification and the art known at the time of the invention that umbilical cord blood mononuclear cells could have been used with the claimed methods*

As noted above, the Examiner acknowledges that an ordinary artisan recognizes that peripheral blood mononuclear cells may be used with the claimed methods. However, the Examiner believes that an ordinary artisan would not have recognized that umbilical cord blood mononuclear cells could also have been predictably used. In contrast to the Examiner's assertions, Applicants submit that an ordinary artisan would have understood from the present application and the art known at the time of the invention that umbilical cord blood mononuclear cells would have been a suitable precursor population for differentiation into cytotoxic lymphocytes.

In support thereof, Applicants submit herewith, D.L. Nelson *et al.*, "The Production of Soluble and Cellular Interleukin-2 Receptors by Cord Blood Mononuclear Cells following *In Vitro* Activation," *Pediatric Research*, 1986, vol. 20, no. 2, pp. 136-139, ("Nelson"), which was published prior to the filing date of the instant application.

Nelson teaches that peripheral blood mononuclear cells *or* umbilical cord blood mononuclear cells may be cultured in a medium containing OKT3, which is an anti-CD3 antibody, to obtain activated cells. Nelson further teaches that IL-2R is increased in activated umbilical cord blood mononuclear cells. Applicants submit that, at the time of the invention, an ordinary artisan would have recognized that IL-2R, which is expressed on the surface of an activated T cell, is a marker for activation. That is, upon expression of IL-2R, cytokine production, cytotoxic activity, proliferation or the like is activated, *see* page 4, lines 18 to 20 and page 25, lines 12 to 15 in the originally filed application. Accordingly, an ordinary artisan would have understood at the time of the invention that IL-2R expression is an indicator of cytotoxic lymphocyte formation. Therefore, an ordinary artisan would have recognized from Nelson that peripheral blood mononuclear cells *or* umbilical cord blood mononuclear cells may be cultured, using the same medium, to obtain cytotoxic lymphocytes.

Applicants further submit that one of ordinary skill would have known, as a matter of fact, that both peripheral blood mononuclear cells and umbilical cord blood mononuclear cells are monocytes, and that these cells have similar characteristics and properties. Accordingly, one of ordinary skill in the art would have readily understood from Nelson and the present application that the production of cytotoxic lymphocytes could have been accomplished by culturing peripheral blood mononuclear cells *or* umbilical cord blood mononuclear cells in the same medium.

The Examiner asserts that anti-CD3 antibody stimulation is not essential to the proliferation and induction of umbilical cord blood mononuclear cells, solely on the basis of Luciverto. However, although Luciverto allegedly teaches that anti-CD3 stimulants fail to induce proliferation of cord blood lymphocytes, an ordinary artisan would have recognized from further art known at the time of the invention, such as Nelson, that cytotoxic lymphocytes may be produced from umbilical cord blood mononuclear cells by anti-CD3 antibody stimulation. Accordingly, Applicants submit that the Examiner supports her contentions using

technical information in an unreasonably narrow manner.

In view of the foregoing, Applicants submit that the present application adequately supports the amended claims. As noted above, an ordinary artisan would have recognized that umbilical cord blood mononuclear cells, as well as peripheral blood mononuclear cells, could have been used with the claimed methods. Accordingly, an ordinary artisan would have also recognized that blood components containing these cells could have also been used. Withdrawal of the rejection is respectfully requested.

**Issues under 35 U.S.C. § 103(a)**

Claims 1-3, 5-7, 10, 12, 28-29, 33-35, and 37-39 are rejected under 35 U.S.C. § 103(a) as allegedly obvious over Jung *et al.*, *J. Immunol.*, 1987, 139:639-644, ("Jung"), in view of Cardarelli *et al.*, *Cellular Immunology*, 1991, 135:105-117, ("Cardarelli"), U.S. Patent No. 5,198,423 to Taguchi *et al.*, ("Taguchi"), Ybarrondo *et al.*, *Immunology*, 1997, 91:186-172, ("Ybarrondo"), and Neri *et al.*, *Clinical and Diagnostic Laboratory Immunology*, 2001, 8:1131-1135, ("Neri"), *see Office Action*, pages 5-8.

Claims 31-32 are also rejected under 35 U.S.C. § 103(a) as allegedly obvious over Jung, Cardarelli, Taguchi, Ybarrondo, Neri, and Chen *et al.*, 1994, *J. Immunol.*, 153:3630-3638, ("Chen"), *see Office Action*, page 8. Applicants respectfully traverse.

*Basis for the Rejection*

The Examiner states that Jung describes culturing PBMCs with anti-CD3 to obtain differentiated cytotoxic CD8+ lymphocytes. According to the Examiner, Jung teaches that culturing PBMCs for 2-3 days results in the greatest cytotoxic lymphocyte activity, which is correlated to a peak in cell proliferation and IL-2 receptor expression. The Examiner admits that Jung does not describe incubating the cells with a recombinant fibronectin fragment. However, the Examiner believes that an ordinary artisan would have combined fibronectin and interleukin-2 with Jung's method since Jung teaches that high levels of proliferation and IL-2 receptor expression correlate with high levels of cytotoxicity and Cardarelli teaches that fibronectin increases cell proliferation.

*Legal Standard for Obviousness*

When considering obviousness of a combination of known elements, the operative question is thus "whether the improvement is more than the predictable use of prior art elements according to their established functions." *KSR International Co. v. Teleflex Inc. (KSR)*, 550 U.S. 398, 82 USPQ2d 1385, 1396 (2007).

*An ordinary artisan could not have predictably achieved the instant invention from the cited references*

Applicants submit that, contrary to the Examiner's assertions, Jung does not teach that cell proliferation is correlated with high levels of cytotoxicity. Jung states that "[c]ytotoxicity rose to maximal levels during 2 to 3 days of stimulation, and this rise correlated with RNA rather than DNA synthesis," *see* Jung, page 642, right column. Jung also states that "[a]lthough induction of RNA synthesis correlated well with that of cytotoxicity, induction of DNA synthesis lagged and correlated with proliferation", *see* page 641, left column paragraph 1, lines 10-13. Since Jung teaches that DNA synthesis is associated with cell proliferation and not cytotoxicity, a person of ordinary skill in the art could not have been reasonably certain that fibronectin, which Cardarelli teaches increases cell proliferation, could also have affected the induction and maintenance of cytotoxicity.

Neither Ybarrondo, Taguchi, Neri nor Chen remedy this deficiency. Ybarrondo pertains to mature cytotoxic lymphocytes and not to the differentiation of PBMCs into cytotoxic lymphocytes. Taguchi and Neri are merely cited for describing SEQ ID NO: 12 and calcein-AM, respectively. Chen is merely cited for describing transduction of a foreign gene into T cells. Accordingly, none of the cited references, either alone or in combination, would have allowed an ordinary artisan to reasonably expect that a substance, fibronectin, which was allegedly described in the art as useful for increasing in cell proliferation could also have been useful for inducing cytotoxicity.

In view of the foregoing, the claims are not rendered obvious by the cited references. Withdrawal of the rejections is respectfully requested.

**Obviousness-Type Double Patenting**

Claims 1-3, 5-7, 10, 12, 28-29, 31-35, and 37-39 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as allegedly unpatentable over claims 1-15 and 20-21 of co-pending Application No. 10/568,745 in view of Jung and Neri, *see Office Action*, pages 8-10. Applicants respectfully traverse.

Applicants submit that the instant application has an earlier filing date than that of U.S. Application 10/568,745. Accordingly, the Examiner should withdraw the rejection in the instant application and address the provisional nonstatutory obviousness-type double patenting rejection in the later filed U.S. Application. According to the MPEP at § 804, if a "provisional" nonstatutory obviousness-type double patenting (ODP) rejection is the only rejection remaining in the earlier filed of the two pending applications, while the later-filed application is rejectable on other grounds, the Examiner should withdraw that rejection and permit the earlier-filed application to issue as a patent without a terminal disclaimer.

**CONCLUSION**

In view of the above amendment and remarks, Applicants believe the pending application is in condition for allowance.

Should there be any outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Linda T. Parker, Ph.D., Registration No. 46,046 at the telephone number of the undersigned below to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Director is hereby authorized in this, concurrent, and future replies to charge any fees required during the pendency of the above-identified application or credit any overpayment to Deposit Account No. 02-2448.

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Respectfully submitted,

By 

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